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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

The Role of Catecholamines in the Development of Pathological Retina Neovascularization in an Experimental Model of Retinopathy of Prematurity in Rats

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Abstract—This work is dedicated to proving our hypothesis that catecholamines and their metabolites play a crucial role in the development of retinopathy of prematurity, which leads to progressive uncontrollable vascularization in the retina, leading to blindness. The study was performed in an animal model of retinopathy of prematurity, which was achieved by hyperoxygenation in rats on postnatal days 7, 14, 21, and 30. The content of catecholamines and their metabolites in the retina of rats was determined by high performance liquid chromatography with electrochemical detection. It was shown that, in the rats with retinopathy, the content of L-DOPA on days 21 and 30 was decreased as compared to the control, whereas the content of noradrenaline on day 14 life increased compared to the control. However, we did not observe changes in the content of dopamine in the experimental animals relative to the control in any period studied. Given the published data on the involvement of catecholamines in the regulation of vasculogenesis in the retina in normal state, our data on the changes in the catecholamine metabolism in the retina in the model of retinopathy of prematurity can be regarded as evidence of the important role of catecholamines in the pathogenesis of this severe disease.

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Retinopathy of prematurity (ROP) is a severe disabling disease that develops in premature infants, is characterized by disruption of normal retinal angiogenesis, and leads in the case of progression to extraretinal growth of abnormal blood vessels with the development of exudative traction retinal detachment. Many studies are devoted to the regulatory mechanisms of retinal angiogenesis in premature infants. However, there is still no clear understanding of which factors exactly trigger the process of abnormal angiogenesis and why in some cases this disease cannot be treated by the existing treatment methods.

In recent years, the angiogenic properties of monoamines are actively studied [1]. A significant part of studies in this field is devoted to tumor neoangiogenesis. It was shown that endogenous dopamine (DA) is an important inhibitor of angiogenesis of a tumor and, as a consequence, of its growth. It was established that DA, acting through D2 receptors, blocks the effects of vascular endothelial growth factor (VEGF) and normalizes the abnormal tumor blood vessels by affecting two cellular component of the vascular wall—pericytes and endothelial cells [1]. Along with this, it was found that noradrenaline (NA) and adrenaline, by influencing β-adrenergic receptors, conversely, stimulate VEGF expression in a number of human tumors [2], which promotes angiogenesis of a tumors and its growth [3].

Given the presence of catecholamines in the retina [4], it is interesting to investigate their role in the regulation of retinal angiogenesis in vasoproliferative diseases of the retina.

The aim of our study was to investigate the role of DA, 3,4-dihydroxy L-phenylalanine (L-DOPA) and NA in the pathogenesis of ROP on the original model of the disease.

The objective of the study was to determine the content of monoamines at different stages of experimental models of ROP.

The study was performed on male Wister rats. To reproduce the experimental ROP, newborn rats together with their mother ($n = 21$) were placed in an incubator for 14 days. Every 12 h the oxygen concentration in the incubator varied from 60 to 15%. Then, the rats were transferred to normoxic conditions (21%). Throughout the experiment, a constant temperature (26°C) and light (12 h day, 12 h night) regimes were maintained. The control group consisted

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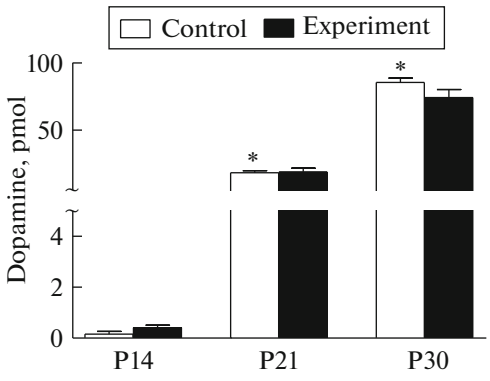

Fig. 1. Content of dopamine in the rat retina in the experimental model of retinopathy of prematurity in ontogeny. P—postnatal development day. * $p < 0.05$ differences in comparison with the previous period of study between the control groups.

of the rats that were kept under normoxic conditions since birth ($n = 20$).

Rats were withdrawn from the experiment on days 7, 14, 21–23, and 30. All pups at the specified time were subjected to binocular enucleation. The retina samples were assayed for the content of NA, DA, and L-DOPA. The isolated retinas were homogenized in 10 volumes of 0.1 N $HClO_4$ comprising 50 pmol/mL DGBA using an ultrasonic homogenizer (Labsonic M, Sartorius) and centrifuged at 16500 *g* for 20 min. The resulting supernatant was assayed for catecholamines.

The compounds were measured by reverse-phase high performance liquid chromatography with electrochemical detection (HPLC-ED) with an LC-20ADsp chromatograph (Shimadzu, Japan). The electrochemical detector DECADE II (Antec, Netherlands) was equipped with a glass carbon flow cell, Ag/AgCl salt bridge, and a reference electrode set at +0.85 V.

Statistical processing of the results was performed using the GraphPad Prism 6 software. The significance of differences between groups with significance level of not less than 95% was evaluated using the nonparametric Mann–Whitney test.

Disruption in retinal angiogenesis in premature infants leads to the development of ROP. To understand the mechanisms underlying this disease, ROP is simulated in newborn animals in which oxygen-induced retinopathy develops due to changes in the concentration of inhaled oxygen. One of the most common animal models of oxygen-induced retinopathy is the model developed in rats, due to the high similarity of the course of this disease with the retinopathy in premature infants.

Taking into account the fact that, in recent years, the angiogenic properties of monoamines are intensely studied, we decided to determine their content in the ROP models that was developed by us earlier [5].

Dopamine. The only source of DA in the retina is the subclass of specialized dopaminergic amacrine cells (DAC). For the first time in ontogeny, these cells containing tyrosine hydroxylase are identified in the rat retina on postnatal (P) days 10–14 [6]. These data are in good agreement with the results of this work. Indeed, DA was also first detected in the retina of rats on day P14; its content was very low, approximately 0.5 pmol (Fig. 1). Later, the content of DA in the retina in normal state (control) increased and, by day P30, increased more than 10 times (Fig. 1), which is consistent with the morphological data on the increase in the number of amacrine cells and their outgrowths [6]. These data indicate that the development of the network of amacrine cells and the formation of their physiological function take place in the main period of the disease—neovascularization and regression of newly formed blood vessels.

In the world literature, there are single works devoted to the determination of DA in rat retina in ROP, and data in them are quite contradictory. For example, Zhang et al. in a rat model and Spix et al. on the mouse model of ROP showed a decrease in the content of DA and its metabolite dioxyphenylacetic acid (DOPAA) in the retina of animals with ROP [7, 8]. The authors demonstrated that, in their models, the DA deficiency was caused by the death of DAC. In our study, the level of DA in the experimental group only tended to decrease ($p = 0.14$) on day P30. This period corresponds to the phase of regression of newly formed vessels. Taking into account the fact that DA can exhibit antiangiogenic activity, it can be assumed that the maintenance of normal physiological level of DA in the retina (at the control level) in the course of neovascularization manifests itself as a compensatory enhancement of the DA synthesis in the survived DAC, aimed at suppressing the uncontrolled angiogenesis. When the growth of blood vessels is ceased and the regression phase begins, the survived cells stop DA hypersecretion, and its level tends to decrease in our experiments. However, this hypothesis requires further investigation.

L-DOPA. In this study, we determined the content of not only DA but also L-DOPA in the retina. In the nervous system, L-DOPA is a precursor of DA and is synthesized from tyrosine with involvement of the enzyme tyrosine hydroxylase. However, L-DOPA is also a precursor of melanin, which is synthesized in the retinal pigment epithelium by the enzyme tyrosinase. Moreover, the GPR143 receptor, whose endogenous ligand is L-DOPA, was found recently in the retina [9]. At the same time, it was shown in human pigment epithelium cell culture that L-DOPA is an antiangiogenic factor causing a decrease in VEGF through the GPR143 pathway [10]. These data allow

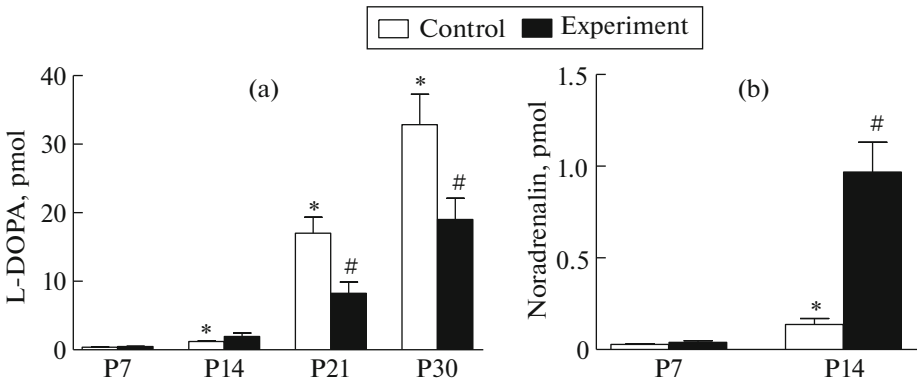

Fig. 2. Content of 3,4-dihydroxy L-phenylalanine (L-DOPA) (a) and noradrenaline (b) in the rat retina in the experimental model of retinopathy of prematurity in ontogeny. P—postnatal development day. Symbols * and # mark the differences in comparison with the previous period of study between the control groups (*) and the differences in comparison with the control (#) at $p < 0.05$.

L-DOPA to be considered the key molecule involved in the ROP pathogenesis.

In our study, L-DOPA was detected in retinal samples in rats in normal state (control) as early as on day P7, in the first period studied (Fig. 2a). Since DA was not detected in samples until day P14, it can be assumed that the main source of L-DOPA in rats in the first two postnatal weeks is the retinal pigment epithelium cells. Interestingly, the level of L-DOPA significantly increases on the third week of postnatal development. Apparently, this is associated with an increase in the number and maturation of both DAC and retinal pigment epithelium cells.

In the experimental model of ROP, we detected no significant changes in the level of L-DOPA in the first three weeks of postnatal development, whereas on day P21 its content in the experimental animals decreased 2 times compared to the control, and this decline was retained on day P30 (Fig. 2). Interestingly, the peak of neovascularization was observed in the period from P18 to P22. Given the fact that L-DOPA inhibits VEGF production in the retina through the GPR143 receptors, and, therefore, exhibits antiangiogenic activity [10], the decrease in its content may contribute to the development of uncontrolled retinal neovascularization.

Noradrenaline. According to published data, NA is a proangiogenic factor that increases VEGF production by affecting β-adrenergic receptors. Moreover, an increase in the synthesis of NA in modeling oxygen-induced retinopathy in mice as well as the efficiency of propranolol in inhibiting neovascularization processes was shown [11]. During the pilot randomized controlled study in the active phase of ROP at the second stage of zone II in children, positive results were obtained in the inhibition of ROP progression by oral administration of propranolol. However, the development of systemic side effects in the form of bradycardia and hypotension was observed [12].

In view of above, in our study we also estimated the level of NA in the ROP model in all periods studied. Although NA was detected in the retina on day P7, no difference in its content between the experimental and control groups was detected (Fig. 2b). On day P14, when, according to the results of our previous studies [5], the expression of proliferating cell nuclear antigen (PCNA) in endothelial cells is detected, indicating the enhancement of their replicative potential, the content on NA in the experimental animals increased by one order of magnitude as compared to the control. Starting from day P21, in this study NA was not detected in the retina of both control and experimental animals. This fact should be given special attention, because the noradrenergic innervation is present in the retina and adrenergic receptors are identified during the entire period of postnatal development [4, 11].

Unfortunately, works devoted to the study of age-related dynamics in the content of NA in the retina are scanty. Shelke et al. detected NA in the retina of rats on day P21; however, for this purpose, they pooled six to eight samples per test [13]. Apparently, the content of free NA in the retina after the second week of postnatal development is quite small and is beyond the resolving power of HPLC-ED. These data allowed us to assume that in the first two weeks of postnatal development, when the content of NA is maximum, it plays a special role in the development of the retina. At the same time, in the course of normal development, the retina undergoes vascularization. On the basis of analysis of our and published data, it can be assumed with a high probability that NA is involved in the normal retinal angiogenesis and plays an important role in the development of ROP. However, this assumption requires further verification.

Thus, our data indicate the involvement of DA, L-DOPA, and NA in the regulation of angiogenesis in ROP and open up broad prospects for the search for new approaches to treatment of this serious disease.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All manipulations with animals were performed in accordance with the protocol approved by the Bioethics Commission of the Koltzov Institute of Developmental Biology, Russian Academy of Sciences and Helmholtz Institute of Ophthalmology in compliance with national and international requirements.

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